

Intrinsically disordered proteins (IDPs) are characterized by high flexibility and low hydrophobic to charged residue ratio. An alanine scan has been conducted along a small molecule binding site of c-Myc, an IDP deregulated in many forms of cancers. This transcription factor undergoes coupled folding and binding with its obligate dimerization partner Max to form a basic helix loop helix leucine zipper. The small molecule, 10058-F4, binds specifically to an 11 amino acid region on the second helix of c-Myc. Binding of 10058-F4 stabilizes the monomeric form of c-Myc and inhibits binding of c-Myc to Max. Residues within the binding site were individually mutated to alanine in order to determine their energetic contribution to the binding of 10058-F4.

Mutation of both hydrophobic and hydrophilic residues attenuates binding of the small molecule to c-Myc. The results support a model in which both hydrophobic interactions and hydrogen bonding are important for binding affinity and specificity in IDP-small molecule interactions.

272-Pos Board B41

Assessing the Coupling in cis between Disordered Regions and Ordered Domains

Anuradha Mittal, Rahul K. Das, Rohit V. Pappu.

Washington University in St Louis, St Louis, MO, USA.

A majority of intrinsically disordered regions occur in cis with ordered domains. This suggests the possibility of functional synergy between ordered domains and disordered regions. Such coupling between distinct modules opens the possibility for increased versatility in protein functions through combinatorial diversity. Despite this, the coupling between ordered domains and disordered regions has been largely unexplored.

Here, we build on our growing understanding of the intrinsic conformational preferences of disordered regions to assess the degree and nature of the modulation of these preferences by cis-acting ordered domains. We use SH3/SH2 domains of NcK adapter proteins and disordered linkers attached to these domains as archetypes of ordered domains in cis with disordered regions. We use atomistic simulations to quantify the intrinsic conformational preferences of different linker sequences. In doing so, we establish that sequence permutants of linkers with similar amino acid compositions can have considerably different conformational preferences. We next use a combination of atomistic and coarse grain simulations to interrogate the nature and degree of coupling between disordered regions and ordered domains. In particular, we quantify and compare the extent of conformational coupling between ordered SH2/SH3 domains for different linker sequences of fixed amino acid compositions and linker sequences with different compositions. We show how insights from the resultant conformational phase diagram are relevant for explaining the phase behavior of copolymers of ordered domains connected by linkers and for heterotypic interactions in signaling pathways that are mediated through synergy between SH2/SH3 domains and their disordered linkers.

273-Pos Board B42

Functional Implications of Intrinsic Helicities within Basic Regions of bZIP Transcription Factors

Rahul K. Das, Kiersten Ruff, Rohit V. Pappu.

Washington University in St. Louis, Saint Louis, MO, USA.

Eukaryotic transcription factors (TFs) including basic leucine zippers (bZIPs) are enriched in disorder promoting residues. In contrast to the presumption that monomeric unbound bZIP basic regions (bRs) are uniformly disordered, recent studies have shown that unbound monomeric bRs have quantifiable helicity. The 8-residue segments, directly N-terminal to DNA-binding motifs are primary modulators of intrinsic helicities. It is conceivable that observed intrinsic helicities and their variation with bR sequences are inconsequential given that sequences of disordered regions change rapidly. Our sequence analysis of bZIPs, however, demonstrates that bRs show a high degree of conservation across orthologs and considerable variation among paralogs. Based on this, we predict that bRs from similar sequence families and different organisms are likely to have equivalent intrinsic helicities and propose that this reflects a mechanistic conservation across orthologs with implications for search process for cognate DNA half sites. To test our predictions we have performed lattice-based kinetic Monte Carlo simulations to quantify the impact of variations in intrinsic helicities on the process of DNA binding. DNA is modeled as a wormlike chain and a randomly chosen site is designated as the cognate site. We model the interplay of three-dimensional diffusion of free TF molecules in solution, binding and unbinding of TF to the non-cognate sites, and one-dimensional sliding along the DNA. The effects of intrinsic helicity on these processes are investigated through systematic titrations in a multidimensional parameter space and quantifying their effects on the first passage time distribution for TF to find its cognate site. The search time has a non-

monotonic dependence on bR helicity such that the search process is optimized within a limited regime of bR helicity. We also quantify effects of bZIP dimerization as a secondary modulator of TF-DNA interactions.

274-Pos Board B43

Quantifying Disorder using Simulated Ensembles for Different Classes of Polypeptides

Nicholas J. Lyle, Rohit V. Pappu.

Washington University, St. Louis, MO, USA.

Intrinsically disordered proteins (IDPs) fail to fold autonomously into singular structures. Molecular simulations, especially when converged, can provide a detailed description of the ensemble of conformations accessible to IDPs. These simulations have shown that IDPs partition into distinct classes such as globules and coils based on their net charge per residue and the classification is obtained using polymeric properties such as average size, shape, and density fluctuations.

Here we complement conventional polymeric descriptors of IDPs with a new measure to quantify the degree of conformational heterogeneity that helps distinguish different IDP classes. This measure is based on the distribution of pairwise similarity between all unique pairs of conformations in the simulated ensemble. Each conformation is converted into an N-dimensional vector of distances, where N equals the number of unique intramolecular pairwise distances. Similarity between pairs of conformations is quantified in terms of the projection between the corresponding pair of vectors. The resultant distribution of conformational (dis)similarities for a given sequence simulated at a specific temperature is calibrated using the canonical Flory random coil. The posterior distribution of conformational (dis)similarities affords a direct measure of (dis) order. The first moment of the posterior (dis)similarity distribution can be used to follow the temperature dependence of conformational properties. This reveals a spectrum of transitions including order-to-disorder transitions and disorder-to-disorder transitions. The latter occurs for two archetypes of IDPs namely, sequences such as polar tracts and highly charged sequences. We also show that variance of the (dis)similarity measure is positively correlated with heat capacities. The new measure of dis(order) is relevant for studying the details of disorder-to-order transitions in coupled folding and binding reactions involving IDPs. It is also relevant for understanding how disorder is maintained in the formation of so-called fuzzy complexes.

275-Pos Board B44

Modulating Neurofilament Sidearm Domain Function by Varying Charge Density

Nithya Srinivasan, Maniraj Bhagwati, Badriprasad Ananthanarayanan, Sanjay Kumar.

University of California, Berkeley, Berkeley, CA, USA.

Neurofilaments (NF) are the most abundant intermediate filaments in large, myelinated neurons and protect the axon against compressive forces. Each filament is surrounded by protruding unstructured protein domains that repel adjacent filaments to drive network assembly. We report the development and characterization of synthetic intrinsically disordered proteins (IDPs) based on the C-terminal sidearm domain of the neurofilament heavy (NF-H) subunit. This domain contains many charged residues and is highly phosphorylated in vivo, and the degree of phosphorylation has been postulated to regulate NF network mechanics. We have expressed three constructs in *E. coli*, and purified them: wild type NF-H; a phosphomimetic NF-H (S to D substitution in the KSP repeats); and a phospho-null polypeptide (S to A substitution in the KSP repeats). The latter two constructs were designed to test the hypothesis that the protein conformation can be modulated by controlling the electrostatic interactions. Dynamic light scattering (DLS) measurements indicate that the recombinant wild type protein adopts an extended conformation with a hydrodynamic radius much larger than that of a globular protein. We demonstrate our ability to microcontact-print these proteins onto a gold-coated silicon wafer using a PDMS stamp, and we verify spatially patterned protein deposition using immunofluorescence imaging. Quartz crystal microbalance studies confirm successful adsorption (0.4 molecules per square nm) of the protein onto gold surfaces. Atomic force microscopy (AFM) reveals that these IDPs can be covalently assembled into Alexander-deGennes polymer brushes, with brush thickness falling with increasing salt concentration. The salt-dependence observed in the AFM data is a direct manifestation of the screening of charge-charge repulsions that modulate the intermolecular interactions. Further studies on the relationship between IDP phosphorylation, solvent conditions, and conformational states should facilitate the exploration of these proteins as building blocks for novel stimulus-responsive biomimetic materials.